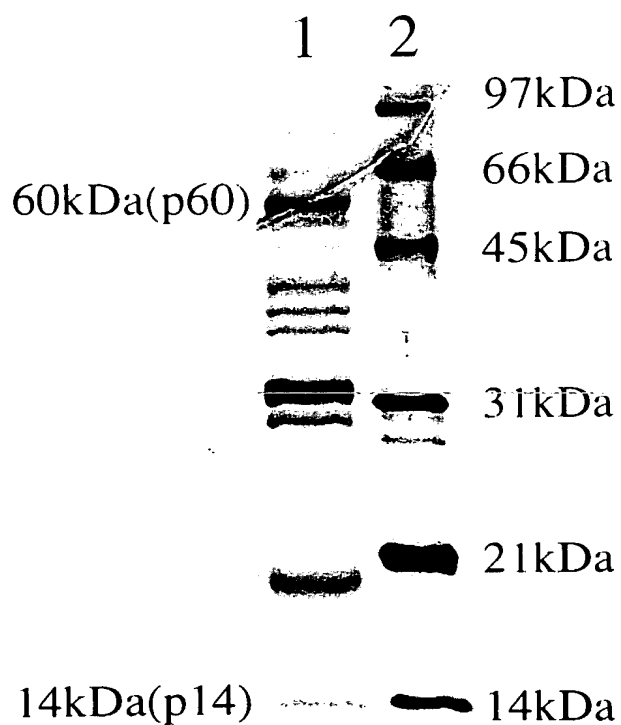


FIG. 1

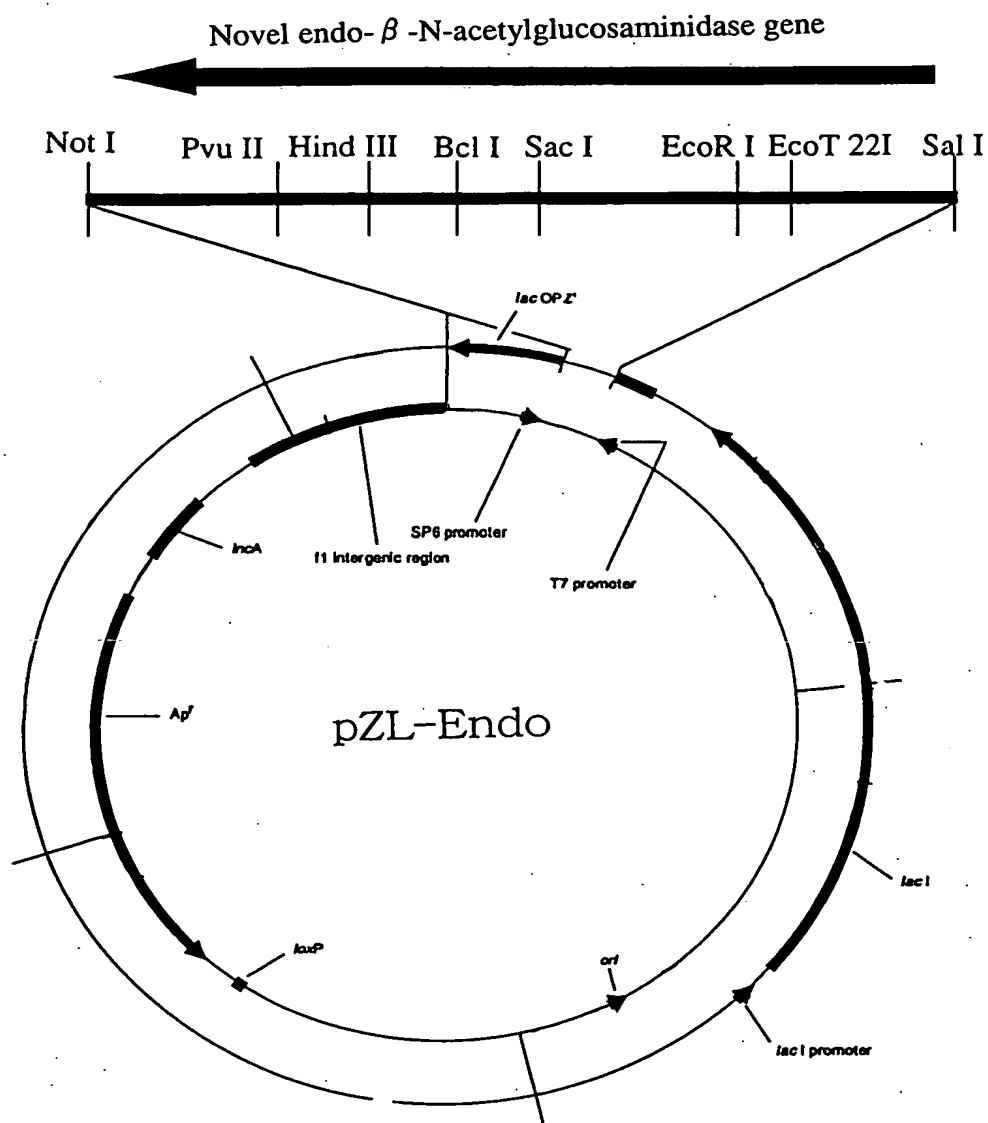


Purification Result for Endo- β -N-acetylglucosaminidase (15-25% gradient SDS-PAGE)

Lane 1: Purified endo- β -N-acetylglucosaminidase from *Mucor hiemalis*

Lane 2: Molecular weight markers

FIG. 2



Restriction enzyme map for pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene.

FIG. 3

10 20 30 40 50 60
 GTTCGACCCAC GCGTCCGCGG ACGCGTGGGC GGACCGGTGG GCGGACCGGT GGGTTTTATT
 70 80 90 100 110 120
 TTACATAAAT ATGCCTTCAC TTCAATTGCA ACCTGATGAC AAACCTAGCAC CTGTTTCTTT
 130 140 150 160 170 180
 TGCACCTAAG TCTATGAATG AGTTGAGGGA CTGGACGCCA GACGAAAAGA TAAAGTTTAA
 190 200 210 220 230 240
 CGTTTCAAGC GTGGCACTAC AGCCTCGTGT GAAAAACGCC CTGAAACCTC AATTATGTGT
 250 260 270 280 290 300
 AACTCATGAT ATGCCAGGAG GATATAAAGA AGATAAAAAT ATTCAAGGAA ACAATTATAA
 310 320 330 340 350 360
 AGACATTAT AACATTCAAT ATTGGCAITTT AGCTGATACT TTTGTATATT TCTCTCATGA
 370 380 390 400 410 420
 GCGAGTTAGC ATTCTCCAG TCAATTGGAC AAATGCTTGT CATAGAAATG GTGTAAAGTG
 430 440 450 460 470 480
 TTTAGGTACT TTTTGTAGTAG AAGGAAATAA CCAAATGCAT GAAATGGAAG CCTTGCTTCA
 490 500 510 520 530 540
 CCGTCCACCT TTAATTAAATA AACTGACGA CCCTATGAGA TTATGGAGTC CGTATTATGC
 550 560 570 580 590 600
 AGACCAATTA GTTGCTATTG CTAAACACTA TGGTTTTGAT GGCTGGTTGT TCAATATTGA
 610 620 630 640 650 660
 ATGCGAATTC TTTCTTTTTC CTACAAATCC AAAATTCAA GCTGAAGAGT TGGCAAAGTT
 670 680 690 700 710 720
 TCTACACTAT TTTAAGGAAA AATTGCATAA CGAAATACCT GGATCTCAAC TCATTGGTA
 730 740 750 760 770 780
 CGACAGCATG ACAAATGAAG GAGAAATCCA CTGGCAGAAC CAGCTCACAT GGAAAAATGA
 790 800 810 820 830 840
 GTTATTTTTT AAAAAACCGG ATGGTATTTT TTTGAATTAT TGGTGGAAAA AAGAATACCC
 850 860 870 880 890 900
 TGAAATGGCG CGTAGAGTAG CTGAAGGAAT AGGTAGATCA GGTTTAGAAG TTTATTTTGG
 910 920 930 940 950 960
 TACAGATGTA TGGGGAAGGC ATACTTATGG TGGCGGTGGT TTCAAATCAT ATAAGGGTGT
 970 980 990 1000 1010 1020
 AAAAACTGCC TACTCTGCAA TGACATCTTC TGCATTATTT GGTATGGCAT GGACATACGA
 1030 1040 1050 1060 1070 1080
 GCATTTTCGAA AAGTCTGAAT TTGAAAAGAT GGATCGTTTG TTTTGGTGIG GTGGTAAATA
 1090 1100 1110 1120 1130 1140
 CTCTGACTAT CCTCCCCAC CTCCTAAAAA CCCAGATGAC GAAAAAGAAG TAGAAAGCGA
 1150 1160 1170 1180 1190 1200
 TGATAGTGAA GATGAGCTCA TGTACGGACA CAAGAAAGGT ATTGCTGACA CGGTAGAATC
 1210 1220 1230 1240 1250 1260
 TATTCCTGTA CCAGGAACAG ATTGGTTTGT TACCAATTTT GATAGGGGGT TTGGAAATAG
 1270 1280 1290 1300 1310 1320
 GTTTTATTAT AGAGGAAAGA GATTACTTTC TCAGCCTTGG TCCCATTTAT CGCATCAAGC
 1330 1340 1350 1360 1370 1380
 TATTCTCCCC AATAAAGCT ATCGAAATCC AGAGATTTAT CCCACTGATC AAAACATTAA

Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene.

FIG. 4

1390 1400 1410 1420 1430 1440
 AATCACTAGT TCTCTCGATT GCGATCATGG AGCTTTTCTT GGTGGAACCT CGCTTATTAT
 1450 1460 1470 1480 1490 1500
 CAAAGGCCAA CGTTTCAATC ATAGAGAATC GCATGATGTT GAAACTGAAA TTAGTATACC
 1510 1520 1530 1540 1550 1560
 TCTGTATAAG CTTTCATTAG ATGCTAGTAA AGGATGCTCA TTGCGTTATA TTTATAGAAC
 1570 1580 1590 1600 1610 1620
 TTTGTGTATG AAAGATGTAA AGTTGACAGT AGCATGTCAC TTTTCGTAA AAACAAACGA
 1630 1640 1650 1660 1670 1680
 CTCAGTTAAT TTCTTCAAGG TATGGCAGCC AGATGAAAAT TTCTCTTTTG AATATGATGA
 1690 1700 1710 1720 1730 1740
 TGGAATGAGA GCCACTGTTA CAACTGAAAA TTCTACCGAA AGCAGATGCT TTTTATTACG
 1750 1760 1770 1780 1790 1800
 TACAACAGAA GAAGATACAG GAGAAAATGA TTGGATAACA AAAACTATTA ATGTGCCTGC
 1810 1820 1830 1840 1850 1860
 TGTTCAGAA GGAAGTCAAT TATACATTAC AAGACTTGAA GTGAGCGTAG TATTAGATAC
 1870 1880 1890 1900 1910 1920
 AGCTGGTTTA GTAGGTCTTG TTAATCAAGT TATTGCTTGC TTGGGATATA TTAGCATCAT
 1930 1940 1950 1960 1970 1980
 ACCAACTATA AATTCCTGGA TAAAAACAGA TTCATCACGC ATTATTACAG ATCTCTTTTG
 1990 2000 2010 2020 2030 2040
 GAAAGATCAG AAATATACCA AAATCGGAAA AGAAAGTTTA GACGACATAG CTCAAGAAGA
 2050 2060 2070 2080 2090 2100
 AGTTCATAGA TATTATGGAA CATTGAACTG GGAAAACACA GCAAATGTAG TAAACGCTTG
 2110 2120 2130 2140 2150 2160
 GGAGGAAATA GATTACTACA ACGTTTTTTT CAAAGAAAGT GACGACTCTG CAACTCGCAT
 2170 2180 2190 2200 2210 2220
 CTTTTTAGGA ACAGCATTCT GTAATCAATT TCGTGTATCT GGTTTAGATA TTATTTTATC
 2230 2240 2250 2260 2270 2280
 TAAGCTACCA AAGATAGTTA TTGAAGCTGT TAACAAAGAA GGATACATCT CTTCAGTGG
 2290 2300 2310 2320 2330 2340
 TAGCATAGAT TTGTCATTAA ACTAGGACTT GAAATAAAAT ATTATGATAA AGAAAAAAA
 2350 2360 2370 2380 2390 2400
 AAAAAAAAAA AAAAAAAG GCGGCCGC.

Entire nucleotide sequence of the fragment inserted into the Sal I-Not I sites of pZL-Endo including the full-length novel endo- β -N-acetylglucosaminidase gene. (Continued)

5'	ATG	CCT	TCA	CTT	CAA	TTG	CAA	CCT	GAT	GAC	AAA	CTA	GCA	CCT	GTT	TCT	TTT	GCA	54
	M	P	S	L	Q	L	Q	P	D	D	K	L	A	P	V	S	F	A	
	CTT	AAG	TCT	ATG	AAT	GAG	TTG	AGG	GAC	TGG	ACG	CCA	GAC	GAA	AAG	ATA	AAG	TTT	108
	L	K	S	M	N	E	L	R	D	W	T	P	D	E	K	I	K	F	
	AAC	GTT	TCA	AGC	GTG	GCA	CTA	CAG	CCT	CGT	GTG	AAA	AAC	GCC	CTG	AAA	CCT	CAA	162
	N	V	S	S	V	A	L	Q	P	R	V	K	N	A	L	K	P	Q	
	TTA	TTG	TTA	ACT	CAT	GAT	ATG	GCA	GGA	GGA	TAT	AAA	GAA	GAT	AAA	AAT	ATT	CAA	216
	L	L	L	T	H	D	M	A	G	G	Y	K	E	D	K	N	I	Q	
	GGA	AAC	AAT	TAT	AAA	GAC	ATT	TAT	AAC	ATT	CAA	TAT	TGG	CAT	TTA	GCT	GAT	ACT	270
	G	N	N	Y	K	D	I	Y	N	I	Q	Y	W	H	L	A	D	T	
	TTT	GTA	TAT	TTC	TCT	CAT	GAG	CGA	GTT	AGC	ATT	CCT	CCA	GTC	AAT	TGG	ACA	AAT	324
	F	V	Y	F	S	H	E	R	V	S	I	P	P	V	N	W	T	N	
	GCT	TGT	CAT	AGA	AAT	GGT	GTA	AAG	TGT	TTA	GGT	ACT	TTT	TTA	GTA	GAA	GGA	AAT	378
	A	C	H	R	N	G	V	K	C	L	G	T	F	L	V	E	G	N	
	AAC	CAA	ATG	CAT	GAA	ATG	GAA	GCC	TTG	CTT	CAC	414	CCA	CCT	TTA	CTT	AAT	AAC	432
	N	Q	M	H	E	M	E	A	L	L	H	G	P	P	L	L	N	N	
	ACT	GAC	GAC	CCT	ATG	AGA	TTA	TGG	AGT	CCG	TAT	TAT	GCA	GAC	CAA	TTA	GTT	GCT	486
	T	D	D	P	M	R	L	W	S	P	Y	Y	A	D	Q	L	V	A	
	ATT	GCT	AAA	CAC	TAT	GGT	TTT	GAT	GGC	TGG	TTG	TTT	AAT	ATT	GAA	TGC	GAA	TTC	540
	I	A	K	H	Y	G	F	D	G	W	L	F	N	I	E	C	E	F	
	TTT	CCT	TTT	CCT	ACA	AAT	CCA	AAA	TTC	AAA	GCT	GAA	GAG	TTG	GCA	AAG	TTT	CTA	594
	F	P	F	P	T	N	P	K	F	K	A	E	E	L	A	K	F	L	
	CAC	TAT	TTT	AAG	GAA	AAA	TTG	CAT	AAC	GAA	ATA	CCT	GGA	TCT	CAA	CTC	ATT	TGG	648
	H	Y	F	K	E	K	L	H	N	E	I	P	G	S	Q	L	I	W	
	TAC	GAC	AGC	ATG	ACA	AAT	GAA	GGA	GAA	ATC	CAC	TGG	CAG	AAC	CAG	CTC	ACA	TGG	702
	Y	D	S	M	T	N	E	G	E	I	H	W	Q	N	Q	L	T	W	

Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence.

FIG. 6

711	720	729	738	747	756
AAA AAT GAG TTA TTT TTT	AAA AAC ACG GAT GGT	738	747	756	
--- --- --- --- ---	--- --- --- --- ---	ATT TTT TTG AAT TAT TCG			
K N E L F F	K N T D G I F L N Y W W				
765	774	783	792	801	810
AAA AAA GAA TAC CCT	GAA ATG GCG CGT	AGA GTA GCT	GAA GGA ATA	GGT AGA TCA	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
K K E Y P E	M A R R V A E G I G R S				
819	828	837	846	855	864
GGT TTA GAA GTT TAT TTT	GGT ACA GAT	GTA TGG GGA	AGG CAT ACT	TAT GGT GGC	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
G L E V Y F	G T D V W G R H T Y G G				
873	882	891	900	909	918
GGT GGT TTC AAA TCA TAT	AAG GGT GTA	AAA ACT GCC	TAC TCT GCA	ATG ACA TCT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
G G F K S Y	K G V K T A Y S A M T S				
927	936	945	954	963	972
TCT GCA TTA TTT GGT	ATG GCA TGG	ACA TAC GAG	CAT TTC GAA	AAG TCT GAA	TTT
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	---
S A L F G M A W T Y E H F E K S E F					
981	990	999	1008	1017	1026
GAA AAG ATG GAT CGT TTG	TTT TGG TGT	GGT GGT AAA	TAC TCT GAC	TAT CCT CCC	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
E K M D R L F W C G G K Y S D Y P P					
1035	1044	1053	1062	1071	1080
CCA CCT CCT AAA AAC CCA	GAT GAC GAA	AAA GAA GTA	GAA AGC GAT	GAT AGT GAA	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
P P P K N P D D E K E V E S D D S E					
1089	1098	1107	1116	1125	1134
GAT GAG CTC ATG TAC GGA	CAC AAG AAA	GGT ATT GCT	GAC ACG GTA	GAA TCT ATT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
D E L M Y G H K K G I A D T V E S I					
1143	1152	1161	1170	1179	1188
CCT GTA CCA GGA ACA GAT	TGG TTT GTT	ACC AAT TTT	GAT AGG GGG	TTT GGA AAT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
P V P G T D W F V T N F D R G F G N					
1197	1206	1215	1224	1233	1242
AGG TTT TAT TAT AGA GGA	AAG AGA TTA	CTT TCT CAG	CCT TGG TCC	CAT TTA TCG	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
R F Y Y R G K R L L S Q P W S H L S					
1251	1260	1269	1278	1287	1296
CAT CAA GCT ATT CTC	CCC AAT AAA	AGC TAT CGA	AAT CCA GAG	ATT TAT CCC	ACT
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	---
H Q A I L P N K S Y R N P E I Y P T					
1305	1314	1323	1332	1341	1350
GAT CAA AAC ATT AAA ATC	ACT AGT TCT	CTC GAT TGC	GAT CAT GGA	GCT TTT CTT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
D Q N I K I T S S L D C D H G A F L					
1359	1368	1377	1386	1395	1404
GGT GGA ACC TCG CTT ATT	ATC AAA GGC	CAA CGT TTC	AAT CAT AGA	GAA TCG CAT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
G G T S L I I K G Q R F N H R E S H					
1413	1422	1431	1440	1449	1458
GAT GTT GAA ACT GAA ATT	AGT ATA CCT	CTG TAT AAG	CTT TCA TTA	GAT GCT AGT	
--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	--- --- --- --- ---	
D V E T E I S I P L Y K L S L D A S					

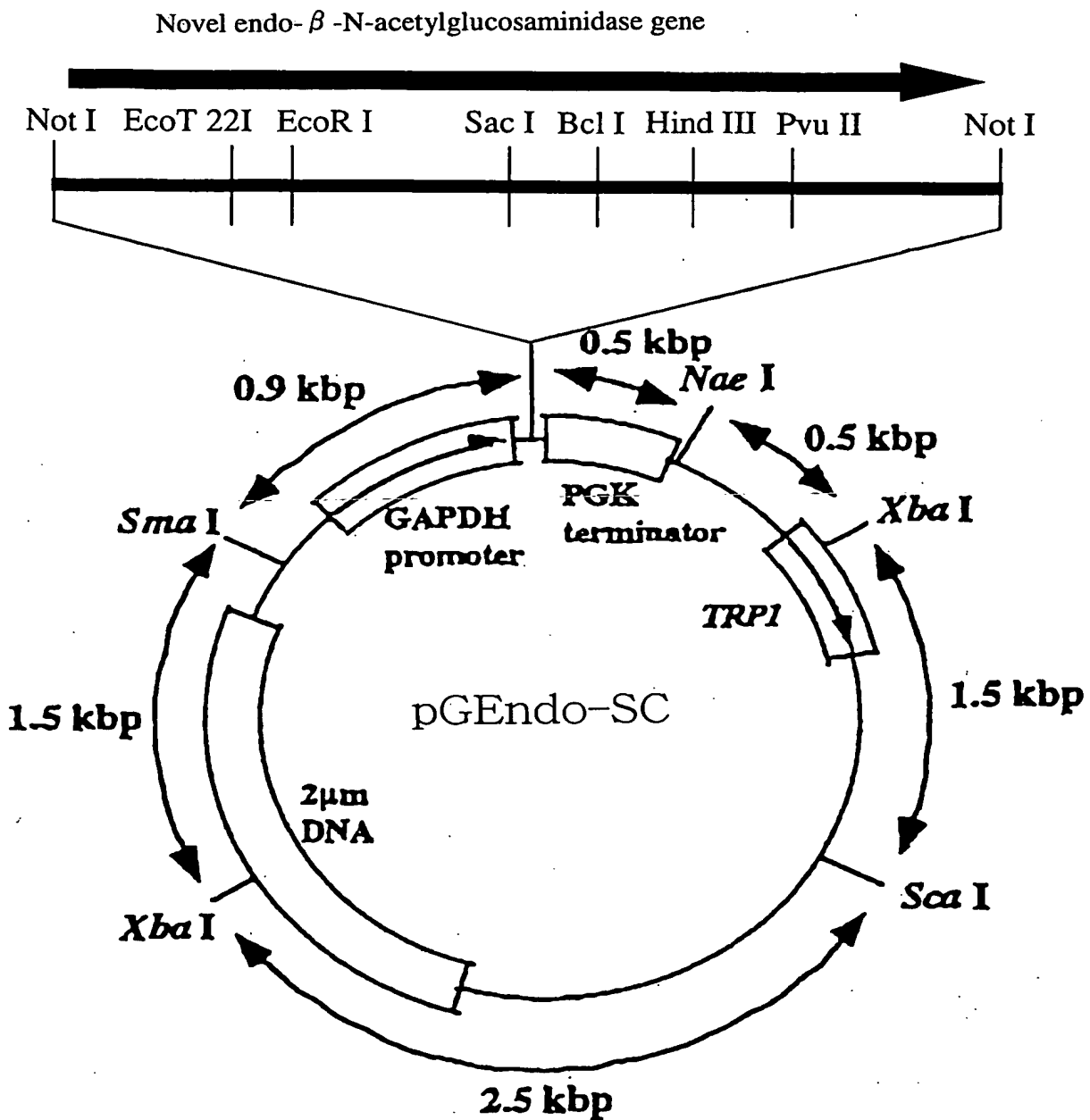
Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence. (Continued)

FIG. 7

1467	1476	1485	1494	1503	1512
AAA GGA TOC	TCA TTG CGT	TAT ATT TAT	AGA ACT TTG	TTG ATG AAA	GAT GTA AAG
K G C	S L R	Y I Y	R T L	L M K	D V K
1521	1530	1539	1548	1557	1566
TTG ACA GTA	GCA TGT CAC	TTT TCG TTA	AAA ACA AAC	GAC TCA GTT	AAT TTC TTC
L T V	A C H	F S L	K T N	D S V	N F P
1575	1584	1593	1602	1611	1620
AAG GTA TGG	CAG CCA GAT	GAA AAT TTC	TCT TTT GAA	TAT GAT GAT	GGA ATG AGA
K V W	Q P D	E N F	S F E	Y D D	G M R
1629	1638	1647	1656	1665	1674
GCC ACT GTT	ACA ACT GAA	AAT TCT ACC	GAA AGC AGA	TCG TTT TTA	TTA CGT ACA
A T V	T T E	N S T	E S R	C F L	L R T
1683	1692	1701	1710	1719	1728
ACA GAA GAA	GAT ACA GGA	GAA AAT GAT	TGG ATA ACA	AAA ACT ATT	AAT GTG CCT
T E E	D T G	E N D	W I T	K T I	N V P
1737	1746	1755	1764	1773	1782
GCT GTT CCA	GAA GGA AGT	CAA TTA TAC	ATT ACA AGA	CTT GAA GTG	AGC GTA GTA
A V P	E G S	Q L Y	I T R	L E V	S V V
1791	1800	1809	1818	1827	1836
TTA GAT ACA	GCT GGT TTA	GTA GGT CTT	GTT AAT CAA	GTT ATT GCT	TGC TTG GGA
L D T	A G L	V G L	V N Q	V I A	C L G
1845	1854	1863	1872	1881	1890
TAT ATT AGC	ATC ATA CCA	ACT ATA AAT	TCT GGA ATA	AAA ACA GAT	TCA TCA CGC
Y I S	I I P	T I N	S G I	K T D	S S R
1899	1908	1917	1926	1935	1944
ATT ATT CAG	GAT CTC TTT	TGG AAA GAT	CAG AAA TAT	ACC AAA ATC	GGA AAA GAA
I I Q	D L F	W K D	Q K Y	T K I	G K E
1953	1962	1971	1980	1989	1998
AGT TTA GAC	GAC ATA GCT	CAA GAA GAA	GTT CAT AGA	TAT TAT GGA	ACA TTG AAC
S L D	D I A	Q E E	V H R	Y Y G	T L N
2007	2016	2025	2034	2043	2052
TGG GAA AAC	ACA GCA AAT	GTA GTA AAC	GCT TGG GAG	GAA ATA GAT	TAC TAC AAC
W E N	T A N	V V N	A W E	E I D	Y Y N
2061	2070	2079	2088	2097	2106
GTT TTT TAC	AAA GAA AGT	GAC GAC TCT	GCA ACT CGC	ATC TTT TTA	GGA ACA GCA
V F Y	K E S	D D S	A T R	I F L	G T A
2115	2124	2133	2142	2151	2160
TTC TGT AAT	CAA TTT CGT	GTA TCT GGT	TTA GAT ATT	ATT TTA TCT	AAG CTA CCA
F C N	Q F R	V S G	L D I	I I L	S K L P
2169	2178	2187	2196	2205	2214
AAG ATA GTT	ATT GAA GCT	GTT AAC AAA	GAA GGA TAC	ATC TCT TCA	AGT GGT AGC
K I V	I E A	V N K	E G Y	I S S	S S G S
2223	2232				
ATA GAT TTG	TCA TTA AAC	TAG 3'			
I D L	S L N	*			

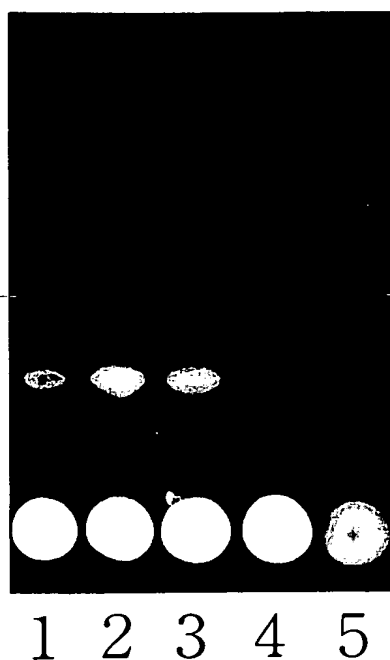
Amino acid sequence deduced from the novel Endo- β -N-acetylglucosaminidase gene, and the nucleotide sequence of the DNA encoding this amino acid sequence. (Continued)

FIG. 8



Structure of expression vector pGEndo-SC for the use in *Saccharomyces cerevisiae*, which comprises a novel endo- β -N-acetylglucosaminidase gene.

FIG. 9



Expression of endo- β -N-acetylglucosaminidase enzyme in yeast into which an endo- β -N-acetylglucosaminidase gene has been introduced.

Lanes 1-3: Cellular extract of *S. cerevisiae* YPH500 (pep4) into which an endo- β -N-acetylglucosaminidase gene has been introduced.

Lane 4: Purified endo- β -N-acetylglucosaminidase derived from *M. hiemalis*

Lane 5: Cellular extract of *S. cerevisiae* YPH500 (pep4)